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Biological Evaluation of Seedling Mortality at DOFAW State Tree Nursery, Kamuela, Hawaii

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ABSTRACT

Excess heat, water stress, and other environmental factors and cultural practices were predisposing seedlings to attack by Pestalotiopsis at the DOFAW State Tree Nursery, Kamuela, Hawaii. No other pathogens were associated with the abiotic-caused loss of over 80,000 seedlings in 1990.

INTRODUCTION

On February 9-12, 1991, Susan Frankel, plant pathologist, visited the Hawaii Division of Forestry and Wildlife (DOFAW) nursery in Kamuela (Waimea), Hawaii to investigate a dieback of nursery seedlings. The trip was in response to a request from Leonard Newell, Pacific Islands forester (USDA-FS), Michael Buck, administrator (DOFAW), and Jeanine Lum, nursery forester (DOFAW). The trip was coordinated by Victor Tanimoto, entomologist (DOFAW).

For the past year and a half, the State Tree Nursery has been trying to overcome a seedling dieback affecting six important tree species. In 1990 the nursery discarded over 80,000 seedlings (nearly 20% of yearly production) due to suspected disease. Pestalotiopsis (syn. Pestalotia), a common secondary or saprophytic fungus, was identified on damaged seedlings. The purpose of this evaluation was to investigate the cause of the seedling damage and determine to what extent other fungi, cultural practices or environmental factors were contributing to the damage.

PROBLEM HISTORY

The State Tree Nursery annually grows 500,000 container seedlings, comprised of over 25 tree species. The seedlings are germinated in a greenhouse that does not have temperature controls and moved outside onto benches after they are a few months old.

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EXPERIMENT STATION

Seedling damage and mortality started to increase in the fall of 1989. Brown needles on lower branches, branch dieback, chlorotic needles, and stunted growth became apparent on seedlings. Fruiting bodies of Pestalotiopsis, (appearing as small black spots) were associated with many of the necrotic areas. Species affected were: Cupressus lusitanica (Mexican cypress), Cupressus macrocarpa (Monterey cypress), Sequoia sempervirens (redwood), Cryptomeria japonica (sugi pine), Pinus radiata (Monterey pine), and Araucaria heterophylla (Norfolk Island pine).

Diseased seedlings were sent to extension plant pathologists at the University of Hawaii. High levels of Pestalotiopsis sp. were identified on the seedlings. Their recommendations included increasing the number of fungicide applications in times of heavy rainfall, and checking for causes of seedling stress.

METHODS

Seedlings, nursery facilities, local environmental conditions and cultural practices were observed and problem history reconstructed.

Soil and plant samples were taken of diseased and healthy seedlings. Foliage and roots were plated onto selective and nonselective media for recovery of plant pathogens. The following media were used: Komada's media (selective for Fusarium) (4); cornmeal + pimaricin media (selective for Phytophthora); V8 juice with pimaricin + antibiotics (selective for Pythium)(5); and potato dextrose agar and water agar (nonselective media).

Seedlings were rinsed in water and as much soil as possible removed from roots. Roots, stems and foliage were chopped into fine segments (<1/4" in length), surface sterilized in 10% chlorox for 30 seconds, rinsed in sterile distilled water, blotted dry and plated onto appropriate media. Soil samples were analyzed by spreading a dilute (5g soil in 100 mls 0.03% water agar) soil solution onto selective medias. Plates were incubated at room temperature in light for 3-7 days (except for media with pimaricin, which was incubated in the dark).

OBSERVATIONS: FEBRUARY 9-12, 1991

Symptoms typical of Pestalotiopsis were present on 4 to 10-month-old seedlings of the following species: Mexican cypress (planted 6-18-90), Monterey cypress (planted 4-4-90, 5-16-90, 8-15-90 and 10-1-90), and Monterey pine (planted 7-90, 10-19-90). The damaged Norfolk Island pine and sugi pine had been discarded previously.

According to Jeanine Lum, nursery forester, seedlings appeared healthier than they did last summer when the suspected disease problem was rampant. Seedlings were being sprayed with mancozeb (Dithane M-45, 1.5 lb/gal, applied every 7 - 10 days), were spaced further apart then previously and dead lower branches had been trimmed off on several crops.

The Mexican cypress displayed the greatest amount of damage typical of Pestalotiopsis. Needle and branch dieback were present, especially in the lower branches. Small black pustules were seen on necrotic areas. Damage was common with over 90% of seedlings showing necrotic areas, but infections were generally limited to only a few branches per seedling. The root systems of affected seedlings appeared normal with adequate fine feeder roots and firm tissues.

Symptoms on Monterey cypress (planted 4-4-90, 5-16-90) were similar, but damage was less frequent with only a few branches dying back. Dead tops or crooked stems with healed-over stem cankers were present on less than 5% of seedlings.

Many of the younger seedlings (less than 4 months old) had different injuries. The younger Mexican cypress (planted 11-30-90) and Monterey cypress (planted 12-6-91) were stunted, swollen and girdled at the base. Approximately 30% of the Mexican cypress and 10% of the Monterey cypress had toppled over due to the weakening at the soil-line and had dead root systems. Less than 5% of the Monterey pine (planted 10-19-90) and Monterey cypress (planted 10-1-90) were showing similar symptoms, being severely stunted with swollen areas and cankers at the base. Estimates of damage from older crops may be underestimates because they had been culled previously.

RESULTS AND DISCUSSION

The seedlings' symptoms, lab analysis results, environmental conditions and cultural practices present at the nursery confirm that stress is predisposing seedlings to infection by Pestalotiopsis. No other pathogens were found to be active at the nursery.

Lab Analysis

No primary pathogens were isolated from soils or plant tissues. Pestalotiopsis was recovered frequently from above-ground portions of 4 to 10-month-old seedlings. It was also visible upon dissecting and compound microscope examination and after incubation in a moist chamber. Penicillium and Botrytis were recovered at low levels and are considered secondary. No other pathogenic fungi were identified on seedlings or isolation plates.

Pestalotiopsis funerea is a saprophyte or weak parasite (2). Under moist conditions, it is found associated with weakened or dead tissues that have been injured by abiotic factors, insects or pathogens. There are other species of Pestalotiopsis (such as P. guepinni) that are considered primary pathogens (11) but these were not detected on seedlings or on isolation plates.

Low levels of potentially pathogenic fungi were found on seedling roots. Only Monterey pine had a high level of Fusarium (100% of pieces plated out) on roots of both healthy and declining seedlings. Since Fusarium was found on roots from healthy and diseased seedlings it is thought to be secondary. Low Fusarium levels were recovered from Monterey cypress and Mexican cypress. No Pythium was recovered from root pieces.

Similar results were found for the soil samples. Fusarium levels in the potting mix were low in all samples tested except Monterey pine. Monterey pine (10-19-90) showed the highest Fusarium level, 610 ppg (propagules per gram), a mixture of F. oxysporum and F. roseum. Pythium levels in the artificial soil were very low. Six of the nine samples showed no Pythium and there was no significant difference between samples taken from healthy or diseased areas.

It is difficult to interpret soil dilution plate results because there are no others from Hawaii to compare them to. However, root pathogen levels in samples taken from potting mix at the base of healthy plants were similar or higher than those taken from diseased areas indicating that root pathogens are not responsible for the damage.

Lab analysis was done in extension plant pathologist Wayne Nishijima's lab at the University of Hawaii, Hilo. Fungal cultures and samples incubating in the moist chamber were discarded after seven days, at the conclusion of the field assistance trip. Some fungi take longer than one week to develop and these may have been missed, although there were no symptoms on seedlings to indicate this was the case.

Nursery Environment and Cultural Practices

The nursery environment and cultural practices are stressing seedlings. Seedings are predisposed to Pestalotiopsis infection by heat stress, uneven irrigation, inadequate nutrition and holding-over of seedlings for longer than is ideal.

Excess heat in the greenhouse environment caused direct and indirect injury to seedlings. Direct heat injury is caused by destruction of plant cells resulting in lesions or necrotic areas on seedlings. Indirect heat injury caused by metabolic disturbance results in reduced growth rate, increased susceptibility to pathogens and other sometimes subtle symptoms (3,7).

Symptoms of direct heat injury were present in the nursery. The younger seedlings (less than 4-months-old) show direct heat injury damage: swelling at the soil-line and girdling of the stem. Soil temperatures greater than 104°F (40°C) have been shown to cause heat lesions on young seedlings which do not have lignified stems, or a canopy for shade to protect them (7).

Heat is building up at the soil-line, where it is absorbed by the black grit top-dressing. Black grit has been replaced with white materials in British Columbia container nurseries after large losses due to heat injury were sustained (Landis, 1991, pers. comm.).

Pestalotiopsis colonizes heat-injured seedlings. The fungus is often associated with a basal girdle of young conifers but fungal parasitism has not been proven. Researchers have concluded that damage is caused by high temperatures rather than fungal infection (8,11).

The older seedlings (4 to 10 months old) may have sublethal, indirect heat damage which is difficult to detect. At elevated temperatures, seedling

proteins are denatured and seedling growth rates are reduced. The exact temperature at which this occurs varies by species and seed source. Redwood is known to be particularly sensitive to heat (7). Recommended temperatures for maximum growth of tree seedlings from end of germination through first weeks of development for redwood are 64-68°F, for Monterey pine 66-72°F (10). Temperature data for other species infected by Pestalotiopsis could not be found. The metabolic disturbance caused by growing at non-ideal temperatures may have predisposed seedlings to Pestalotiopis infection.

In the fall of 1989 the greenhouse panels were replaced with Lasco Crystalite-T panels and 6 months later, additional screening was installed on the sides to protect seedlings from wind and birds. A side effect of these modifications has been to increase temperatures in the greenhouse. The panels transmit over 50% more light than the old ones, increasing the heat, and the screening reduced the wind, thereby reducing convection and conduction of heat away from seedlings. The outbreak of Pestalotiopsis occurred shortly after the time these alterations were made.

No greenhouse air or soil temperature data was available for 1989 and 1990, but the Lalamilo Irrigation District field office adjacent to the nursery had climatological records of outdoor air temperatures. The records show that temperatures in Kamuela are fairly consistent year round with August to November being slightly warmer. The average high temperature from August to November 1990 was 76.8°F , and average minimum was 58.6°F. Greenhouse air and soil-surface temperatures are estimated to be much higher. Workers have complained of being uncomfortably hot in the greenhouse.

Intense solar radiation may be contributing to the problem as well. The nursery is located at approximately 2500 feet elevation and a tropical latitude. Solar radiation can be intense under these conditions. To avoid burning, shade is recommended for transplanted pine seedlings and seedbeds in tropical areas and must be removed gradually to avoid undue stress due to sudden exposure to sunlight (6).

Insufficient nutrients and uneven irrigation are also contributing to predispositon of seedlings to Pestolotiopsis infection. Winds which prevail at Kamuela prevent irrigation water from reaching the containers evenly. Average wind speed in the area (in 1989), was 17 mph with daily recordings of 80 mph not uncommon. Water is blown off-target between the time it emerges from overhead nozzles and reaches the level of containers located approximately 5 feet below. The nursery was originally designed as a bareroot nursery and was converted to a container nursery in 1977. The water system was designed for the bareroot nursery and the pressure it develops is not ideal for distributing water in a container nursery. The nursery manager has been working on modifying the water system to improve distribution of water. As continued improvements are made seedling health should improve as well.

After visits to the nursery, Tom Landis, USDA-FS western nursery specialist and Wayne Nishijima, extension plant pathologist, University of Hawaii at Hilo, both independently concluded that inadequate seedling nutrition was stressing seedlings at the nursery. Based on seedling color and reduced growth rate, Landis concluded that the liquid fertilizer was not being taken-up by the

seedlings (Letters, Landis 12/14/88, 6/9/89). Nishijima took weak koa seedlings and exposed them to nutrient solutions in the laboratory. Seedlings responded by greening-up and improving in overall appearance (Nishijima, 1991, pers. comm.). Improvements in fertilization regime have been made in 1989 and 1990, but inadequate fertilization still appears to be stressing seedlings at the nursery.

Due to the windy conditions, fertilizer can not be distributed through the irrigation system. Instead, it is hand applied using a tractor-pulled tank and hose. This is a time-consuming, labor-intensive operation which limits the frequency of fertilizer application. Liquid fertilizers are used with various amounts applied to different species. Slow release fertilizers are not used due to the difficulty of getting even amounts of fertilizer into small containers and corrosion of the soil mixer. A stainless steel mixer has been ordered for the nursery and this should eliminate the corrosion problem. Adequate fertilization is necessary but over fertilization needs to be avoided because it prevents hardening-off of seedlings and causes seedlings to grow too fast. Nutrient rich tissues are also highly susceptible to pathogens.

Holding seedlings in the nursery for a longer time than ideal may also be contributing to the problem. The held-over stock becomes root-bound, stressing seedlings and making them vulnerable to pests such as Pestalotiopsis. The need to hold seedlings longer in the nursery is often unavoidable due to uncertainties in planting needs and logistical problems.

Other factors that may be contributing to the damage and mortality are a lack of mycorrhizae, and lack of a seed testing and seed source tracking program. These are areas that the nursery may want to develop in the future after the primary needs of even irrigation and adequate seedling nutrition are met.

RECOMMENDATIONS

Continue preventive mancozeb fungicide sprays. Pestalotiopsis is found on seed, in air, and in soils. It is ubiquitious and therefore difficult to control by improving sanitation. To slow development of fungal resistance to mancozeb, alternate fungicides. Fungicides such as chlorothalonil and Bordeaux mixture applied to the foliage every 2 weeks have provided control of Pestalotiopsis on white pine in the Southeastern United States (1).

Set up small field trials to determine survival and quality of Pestalotiopsis infected seedlings after outplanting. Keep records of level of Pestalotiopsis infection at planting and watch for increase or decrease over time. It is thought that seedlings can be successfully outplanted if Pestalotiopsis infection is limited on planting stock. If this is done at the nursery, locate planting as far as possible from crop trees so it does not increase spore loads at the nursery. Pestalotiopsis is found throughout Hawaii and you need not be overly concerned about spreading the fungus into new areas.

Monitor greenhouse air and soil temperatures to correlate heat stress with seedling condition. Dial thermometers placed immediately below the soil surface could be set up in a few locations. In addition, maximum and minimum

daily air temperatures need to be monitored inside the greenhouses, and outside in full sun and shade.

Use sprinkler irrigation, applied during the hottest portion of the day, to cool soils, and seedlings. This will also hasten drying of foliage after irrigation thereby decreasing opportunities for fungal infection. Observations of seedlings' responses to heat need to be made and guidelines for cooling developed.

Change from black grit top-dressing to white. This will reduce heat absorption and retention. Shading would also reduce temperatures, but is expensive to install and can be labor-intensive. Irrigation and changing top-dressing color should adequately protect seedlings from heat.

Continue to test slow release fertilizers as a supplement to liquid fertilizer. Continue to experiment with irrigation and fertilizer application methods to improve even distribution to plant roots. The installation of windbreaks, that is currently underway should help improve water and fertilizer distribution.

Contact Susan Miyasaka and Dr. Mitiku Hibte at the University of Hawaii, Manoa to learn more about mycorrhizae in tropical areas. Mychorrizae levels were not determined in this analysis. A lack of mychorrizae may be contributing to inadequate seedling fertility.

CONCLUSIONS

Continuing to improve seedling quality will reduce Pestalotiopsis damage in the nursery. No other pathogens were found, therefore, enhancing seedling nutrition and irrigation, and protecting seedlings from heat and wind should reduce Pestalotiopsis infection to minor, tolerable levels.

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